structural basis for permeation, drug-block, and voltage-dependent gating. Voltage-sensing depends on the S4 segments, which move outward through a gating pore via a 'sliding-helix' mechanism. Outward movement of S4 is coupled to pore-opening by tilting and rotation of the S6 segments. A structural model reveals conformational changes underlying activation and pore-opening, and disulfide locking of substituted cysteines demonstrates voltage-dependent formation of ion pairs during channel activation, as in the sliding-helix model. Na+ channel blocking drugs bind to a conserved receptor site in the inner pore, and binding is enhanced by repetitive opening of the pore and inactivation of the channel. A three-dimensional view of these structural components is provided by a new high-resolution structure of a voltage-gated Na+ channel from Arcobacter butzleri (NavAb) captured in a closed-pore conformation with four activated voltage-sensors at 2.7 Å resolution. The arginine gating charges make multiple hydrophilic interactions within the voltage-sensor, including unanticipated hydrogen bonds to the protein backbone. Comparisons to previous open-pore K+ channel structures suggest that the voltage-sensor domains and the S4-S5 linkers dilate the central pore by pivoting around a hinge at the base of the pore. The NavAb selectivity filter is short, ~4.6 Å wide, and water-filled, with four acidic glutamate side-chains surrounding the narrowest part of the ion-conduction pathway. This unique structure presents a high-field-strength anionic coordination site, which confers Na+ selectivity through partial dehydration via direct interaction with glutamate side-chains. Fenestrations in the sides of the pore are unexpectedly penetrated by fatty-acyl chains that extend into the central cavity, and these portals are large enough for entry of small, hydrophobic pore-blocking drugs. Supported by NINDS/NIH.

Subgroup: Exocytosis & Endocytosis

40-Subg

Vesicle-Membrane Fusion is Enhanced by Cholesterol and Low Temperature

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Lipid composition and properties play an important role in fusion of vesicles to cell membranes, an essential process for exocytosis. Using a model system composed of artificial vesicles and planar lipid membranes, we observed that fusion is significantly affected by the lipid phase of the planar membrane. To specifically determine the effect of lipid phases on fusion rates, we utilized the nystatin/ergosterol fusion assay and stimulated fusion with an osmotic gradient. The lipid phase of the planar membrane was altered by changing cholesterol or temperature, while the vesicular lipids were held constant. Liquid disordered (L_α) planar membranes were formed from PE and PC. Addition of cholesterol shifts these membranes to the liquid ordered (L_o) phase and increased vesicle fusion. Planar membranes in the L_α phase were also made from DPPC lipids above the transition temperature (41.5°C); decreasing the temperature shifts these membranes into the ripple phase ($P_{\beta'}$) and again increased vesicle fusion. These data are consistent with the hypothesis that fusion is promoted in membranes with some negative curvature.

41-Subg

Existence of a Novel Clathrin-Independent Endocytic Pathway in Yeast

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Yeast is a powerful model organism for dissecting the temporal stages and choreography of the complex protein machinery during endocytosis. The only known mechanism for endocytosis in yeast is clathrin-mediated endocytosis, even though clathrin-independent endocytic pathways have been described in other eukaryotes. In my talk, I will present evidence for a clathrin-independent endocytic pathway in yeast. In cells lacking the clathrin-binding adaptor proteins Ent1, Ent2, Yap1801 and Yap1802, we identified a second endocytic pathway that depends on the GTPase Rho1, the downstream formin Bni1, and the Bni1 co-factors Bud6 and Spa2. This second pathway does not require components of the better-studied endocytic pathway, including clathrin and Arp2/3 complex activators. Thus, our results reveal the existence of a second pathway for endocytosis in yeast, suggesting similarities with the RhoA-dependent endocytic pathways of mammalian cells.

Subgroup: Permeation & Transport

42-Subg

Single-Molecule Transport Across an Individual Biomimetic Nuclear Pore Complex

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Nuclear pore complexes regulate the selective exchange of RNA and proteins across the nuclear envelope in eukaryotic cells. Biomimetic strategies offer new opportunities to investigate this remarkable transport phenomenon.
In this talk, I will show selective transport of proteins across individual biomimetic nuclear pore complexes at the single-molecule level. Each biomimetic complex is constructed by covalently tethering either Nup98 or Nup153 (phenylalanine-glycine (FG) nucleoporins) to a solid-state nanopore. Individual translocation events are monitored using ionic current measurements with sub-millisecond temporal resolution. Transport receptors (ImpB) proceed with a dwell time of ~2.5 ms for both Nup98- and Nup153-coated pores, whereas the passage of non-specific proteins is strongly inhibited with different degrees of selectivity. For pores up to ~25 nm in diameter, Nups form a dense and low-conducting barrier, whereas they adopt a more open structure in larger pores. Our biomimetic nuclear pore complex provides a quantitative platform for studying nucleocytoplasmic transport phenomena at the single-molecule level in vitro.
* Work carried out by my graduate student Stefan W. Kowalczyk at Delft and in collaboration with Larisa Kapinos and Roderick Y. H. Lim at the University of Basel.

43-Subg

Identification and Structure Determination of a Bacterial Hydrosulfide Ion Channel

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Though it is believed to have been critical to the origin of life on Earth, hydrogen sulfide (H2S) still plays a prominent role in physiology and cellular signaling. Its undissociated form, hydrosulfide ion (HS-), is a major metabolite in bacterial anaerobic fermentation. While toxicity due to HS- and H2S, has been extensively characterized, the molecular mechanism for HS- transport across the cell membrane is unknown. Through a combination of genetic, biochemical and functional approaches, we have identified a hydrosulfide ion channel (HSC) in the pathogen Clostridium difficile. The HS- channel is a member of the formate-nitrite-transport (FNT) family, and ~50 HSC genes form a third subfamily besides those for formate and for nitrite. In addition to HS- ions, HSC is also permeable to formate and nitrite. Crystallographic studies revealed an HSC structure that is similar to that of the formate channel FocA [1], with an aquaporin fold that is arranged into a pentamer. Structural and biochemical characterization showed that the ion-selectivity filter is located in the center of the protomer.
Reference:
1. Waight AB, Love J, Wang DN: Structure and mechanism of a pentameric formate channel. Nat Struct Mol Biol 2010, 17:31-37.